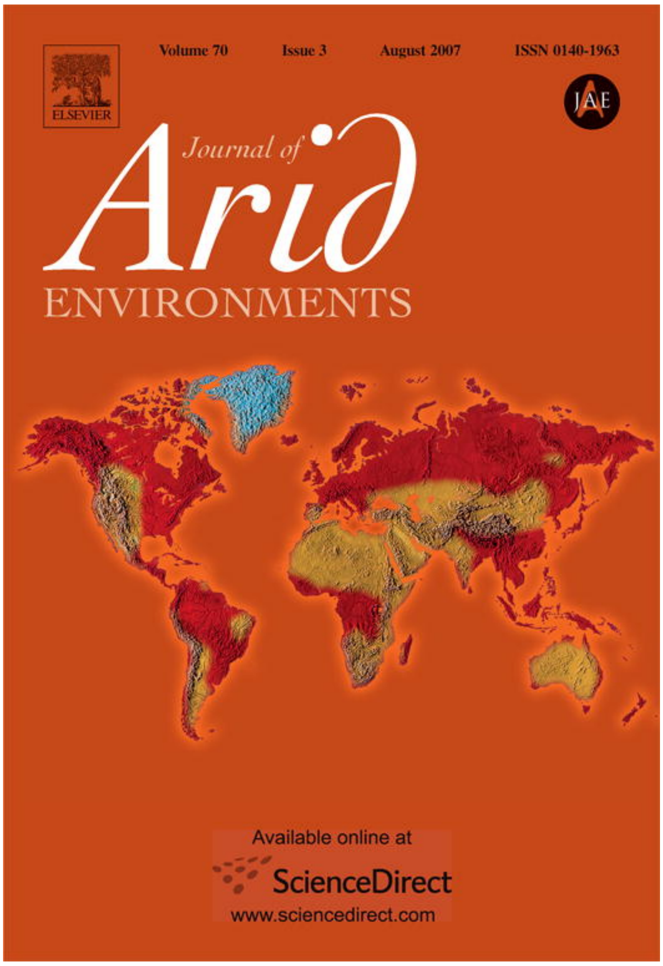
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Short communication

Effect of heat and ash treatments on germination of *Pinus pinaster* and *Cistus laurifolius*

C. Herrero[a,*], R. San Martin[b], F. Bravo[a]

[a]*Departamento de Producción Vegetal y Recursos Forestales, E.T.S. de Ingenierías Agrarias, Universidad de Valladolid, Avda de Madrid, 44, 34004 Palencia, Spain*

[b]*Departamento de Estadística e Investigación Operativa, E.T.S. de Ingenierías Agrarias, Universidad de Valladolid, Avda de Madrid, 44, 34004 Palencia, Spain*

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Abstract

Two experiments were performed to study the effect of fire and ash concentration on the germination of *Pinus pinaster* and *Cistus laurifolius* seeds. These experiments combined seven different temperatures, ranging from 70 °C up to 190 °C, and two times of exposure, 1 and 5 min. In addition to these treatments, a control treatment was performed in the absence of thermal shock. Following the heat treatments, seeds were introduced into a germination chamber in a randomized design and under controlled conditions of temperature and photoperiod.

P. pinaster seeds responded positively in germination to thermal shocks of short duration (1 min) over the studied range of temperatures. Nevertheless, in the simulation of fires of long duration, temperatures equal to or higher than 130 °C, resulted in lethal effects on seeds. On the other hand, *C. laurifolius* seeds responded positively if the temperature was below 110 °C, independent of time of exposure. The effect of ash concentration was also tested but failed to produce significant differences in the germination rates of either species.

The results of this study suggest prescribed fire could be incorporated in sustainable forestry practices following detailed studies of its direct and indirect effects.

Keywords: Competition; Fire; Pine; Probability; Regeneration; Seed; Thermal shocks

*Corresponding author. Tel.: +34 979 108427; fax: 34 979 108440.
E-mail address: chdeaza@pvs.uva.es (C. Herrero).

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1. Introduction

Post-fire regeneration is a complex process dependent upon factors such as germination strategies of species, their characteristics and adaptations to fire, the abundance of seeds in both soil and aerial banks, the conditions of the seedlings (light, temperature and moisture conditions, seed protection to predators), the fire parameters (time of year, size, severity), etc. (Tapias et al., 2001; Thanos et al., 1989; Trabaud et al., 1985).

Fire intensity is one of the most important factors influencing post-fire regeneration of an ecosystem (Malanson, 1984; Sousa, 1984). Fire intensity is characterized by two factors: time of exposure and temperature reached during the fire (Gill and Groves, 1981; Moreno and Oechel, 1991; Núñez and Calvo, 2000). Both greatly influence the capacity of plant communities to recover after fires. Other important factors include competition from opportunistic species, whose seed dormancy can be broken under certain temperatures, and the effect of ash deposited on the ground, (Neéman et al., 1993; Reyes and Casal, 2006; Thomas and Wein, 1990), which may promote the release of large amounts of nutrients necessary for plants (Reyes and Casal, 1998).

To evaluate the germination response after different fire intensities, thermal shocks stand out among the most widespread techniques to break seed dormancy and thus spur germination (Núñez and Calvo, 2000; Thanos et al., 1989; Valbuena et al., 1992). Thermal treatments may be carried out using dry heat where seeds are heated in an oven at different controlled temperatures, using a range of temperatures simulating the conditions that exist in the soil during a natural fire, while combining different exposure times (Pérez and Pita, 1996).

Different previous studies have verified that the heat that acts in a fire on a concrete point operates during a short lapse of time (between five and 15 min), and it reaches a range of temperatures between 44 and 150 °C, to 2.5 cm of depth (Trabaud, 1979). Therefore, a wide range of temperatures includes a lot of possibilities contrasted with studies of prescribed fires, where similar temperatures were reached in the soil (Hernando et al., 2000), and in the crown, where the maximum reached temperatures did not exceed the 140 °C (Vega, 2000).

The germination response to temperature and exposure time varies depending on the species or even the origin of the seed population (Pérez and Pita, 1996), however, the range of temperatures and time exposure used by former researchers was between 50 and 70 °C as a lower limit and 190–200 °C as an upper limit (Clark and Wilson, 1994; Núñez and Calvo, 2000; Villalobos et al., 2002).

Pinus pinaster Ait. (Oria de Rueda and Díez, 2003) is a common tree species, distributed throughout coastal regions in the Mediterranean basin of Europe and Africa, the Atlantic coasts of Portugal, Spain and France, as well as at mid-altitudes on mountain ranges and inland plains of the Iberian Peninsula. *P. pinaster* is also a species greatly affected by forest fires. In Castile-Leon (Spain), the statistics on burnt area by species in the period 1993–2001 revealed that *P. pinaster* was the second most affected species, at 15.926 ha (5% of its distribution). In relation to wildfires, along with *Pinus halepensis*, *P. pinaster* has been characterized as a typical pyrophite, because it regenerates well after a forest fire (Castro et al., 1990; Núñez and Calvo, 2000).

Cistaceae is essentially a circum-Mediterranean family, with its highest level of diversity in the Western part of its range, mainly the Iberian Peninsula. According to Warburg (1980), 12 species of *Cistus* occur in the Iberian Peninsula, of which *Cistus* *laurifolius* join with *Cistus albidus*, *Cistus ladanifer*, and *Cistus salvifolius* are remarkable representatives.

Cistus spp. are markedly heliophylous shrubs species with a colonizer behavior on highly degraded areas. Germination strategy is based on producing a large crop of seeds per year. *Cistus* seeds revealed the presence of a dormancy imposed by the seed coats; therefore, fire is necessary for this colonization to take place (Arianoutsou and Margaris, 1982; Montgomery and Stird, 1976). High temperatures from thermal shocks are effective in breaking dormancy (Viullemin and Bulard, 1981).

The main goal of this paper is to evaluate the effect of fire through temperature and exposure time on the germination of *P. pinaster*. The effect of fire on the species *C. laurifolius*, a species that is potentially a competitor of *P. pinaster*, is also studied. Finally, the effect of ash concentration on the germination of these two species is analyzed.

2. Materials and methods

Two experiments were carried out in order to achieve the proposed objectives.

2.1. Experiment I

P. pinaster seeds were obtained from the Spanish Forest Genetics Service and from the "Castilian Plateau" region (Central Spain), and were harvested in 1998–99. Up to the time of the experiment, the seeds were stored in opaque paper bags at a constant temperature (4 ± 2 °C). Their germination potential was thus homogenized avoiding internal seed factors, which might condition their response to treatments.

Fifteen treatments were performed, resulting from a combination of seven different temperatures (70, 90, 110, 130, 150, 170 and 190 °C) and two exposure times (1 and 5 min) plus a control treatment with no thermal treatment. The control treatment was considered as 20 °C (Hanley and Fenner, 1998) and 1 min of time of exposure in later statistical process. Thermal shocks were performed in a hot dry oven. Five replications were used. Each experimental unit was made up of 20 seeds placed on a 9-cm Petri dish with two discs of filter paper moistened with de-ionized water. The seeds averaged 0.05 g in weight and were approximately 10–15 mm^3 in size, so ample room was available for each seed on the disc. After treatment, Petri dishes were randomly placed into a germination chamber and kept for 6 weeks under constant conditions of 21 °C in temperature for 14 h with white light and 17 °C for 10 h in the dark. A seed was regarded as germinated when the radicle emerged 2 mm from the testa (Come, 1970) and was removed.

A one-way analysis of variance (ANOVA) was carried out for the 15 treatments considered, in which the dependent variable was the arcsine of the square root of the proportion of seeds germinated on each dish. This transformation was used to correct the statistics' assumptions underlying the normality of the linear model (Montgomery, 1997). In order to detect significant differences between the treatments, Tukey's multiple range test was used.

2.2. Experiment II

As in Experiment I, *P. pinaster* and *C. laurifolius* seeds were obtained from the Spanish Forest Genetics Service. *P. pinaster* seeds came from the harvest of 1998/99, while

C. laurifolius seeds were from 2000. Both species' seeds were stored in opaque paper bags at a constant temperature ($4 \pm 2\,^{\circ}C$) until the experiment was performed.

The seeds of both species were subjected to the same thermal treatments used in the first experiment. *C. laurifolius* seeds were roughly one-fifth of that of *P. pinaster* seeds (3–5 mm^3 approximately). Experimental units consisted of 20 seeds placed on Petri dishes with filter paper moistened with de-ionized water as in the first experiment. In order to evaluate the effect of ash concentration on the germination of these two species, the seeds on the Petri plates were sprinkled with three solutions: two with 1 and 5 g/l ash and a third with distilled water. The ash was obtained by burning a sack of pine needles and branches of *P. pinaster* gathered from a pine stand in Central Spain. Once burnt, the remains were kept in a dry environment and sifted in order to make it easier to dissolve.

In all, 90 treatments were analyzed in this test, resulting from the same 15 thermal treatments as in Experiment I, three different ash solutions and two species. Each of the treatments was repeated three repetitions, and the 270 experimental units were placed in the controlled-environment germination chamber at random, under the same conditions of temperature and lighting duration as in the first test, i.e., constant conditions of 21 °C in temperature for 14 h with white light and 17 °C for 10 h in the dark. The experiment also lasted 6 weeks. *C. laurifolius* and *P. pinaster* seeds were regarded as germinated when the radicle emerged 2 mm from the testa and was removed (Come, 1970).

The analysis of a complete factorial experiment for the factors of species, thermal treatment and ash concentration was carried out, including the three factors and all of their possible interactions. In addition, to create a model of seed germination likelihood, a logistic model was formulated including the considered factors and their interactions as independent variables. The two species were placed in the model using a variable dummy (one if *P. pinaster*, zero otherwise). The Stepwise Variable Selection method was applied using input and output criterion $\alpha = 0.05$. All of the analyses were carried out using the SAS 8.1 statistical program (SAS Institute Inc., 2001).

3. Results

In Experiment I significant differences were obtained ($F = 39.65$, $p = 0.0001$) among the different thermal treatments. The analysis showed a determination coefficient of 90.2% and a variation coefficient of 20.5%. Temperatures greater than or equal to 130 °C were more sensitive to exposure time, especially when the exposure time was equal to 5 min (Fig. 1).

On the other hand, when exposure time was 1 min, germination was less variable, without significant differences except at a temperature of 190 °C, where germination was reduced slightly (Fig. 1).

In Experiment II, the analysis of the effect of temperature, exposure time and ash concentration ($F = 16.76$, $p < 0.0001$) showed that it was a highly significant model, with a determination coefficient of 89.2% and a variation coefficient of 22.0%. The factors of species, thermal treatment and their interaction were significant at $\alpha = 0.05$. Not significant was the ash factor or its interactions with heat and species. Therefore, the results showed that the influence of heat on germination displayed statistically significant differences between the two species and amongst treatments. By plotting the interactions (Fig. 2), it could be observed that, for *P. pinaster*, the germination rates did not display statistically significant differences between thermal treatments at 1 min, whereas at 5 min,

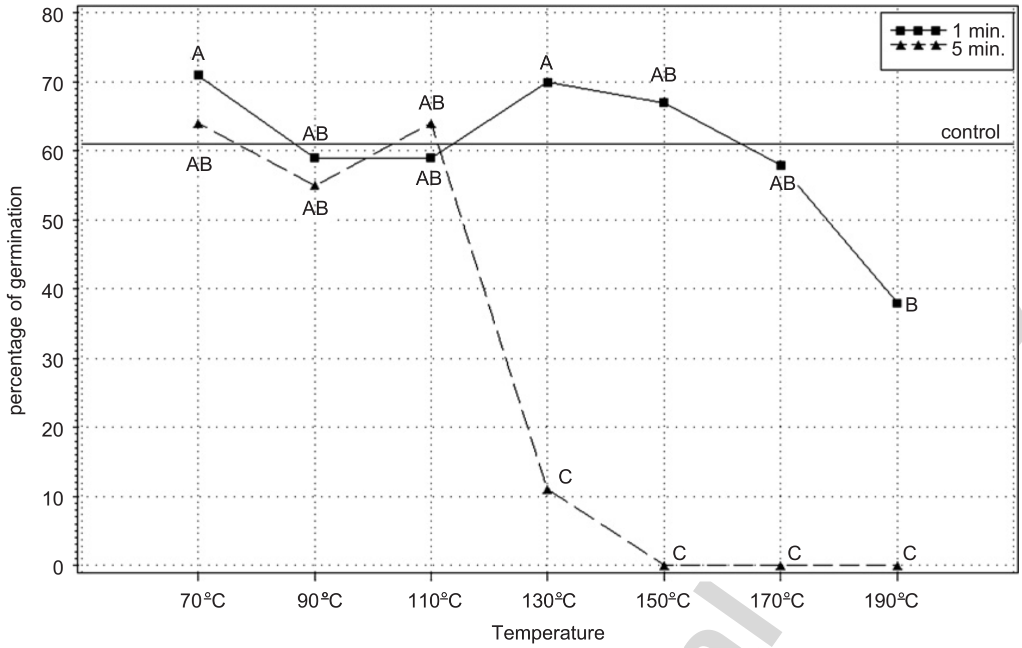

Fig. 1. Percentage of seeds successfully germinating of *Pinus pinaster* after studing the effect of thermal shocks and Tukey's test groups.

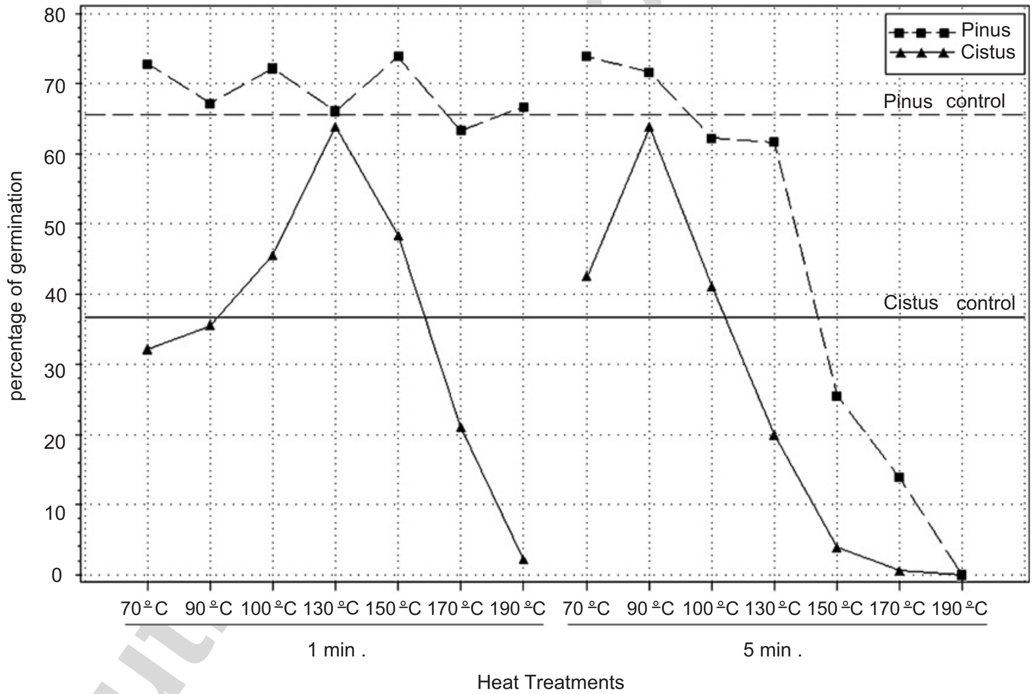

Fig. 2. Percentage of seeds successfully germinating of *Pinus pinaster* and *Cistus laurifolius* after studing the effect of thermal shocks and ash concentration in both species.

for temperatures above 130 °C, there was a significant decrease in these rates, as the results of Experiment I, previously showed. The germination rates of *C. laurifolius* for 1 min rose with temperature up to 130 °C, but after that significantly dropped. In thermal treatments at 5 min, the behavior was similar to *P. pinaster* but a maximum germination rate was reached at 90 °C.

The logistic regression model provided additional information on the differences between species and the influence of the different factors considered in the study on germination rates. In addition, this method allowed the introduction of quadratic terms in the logistic process to check how they could affect the probability of germination. It could be seen that the ash concentration in interaction with species and in triple interaction with species and time of exposure influenced the probability of germination, though in variance analysis they were not significant factors. After the procedure to select variables, the resulting model had a Wald's statistical value χ^2 of 918.518 with nine df, with p-value <0.0001 for testing the global null hypothesis $H_0{:}\beta = 0$. The final model with the selected variables was as follows:

$$\ln\left(\frac{p}{1-p}\right) = -2.6978 + 2.2888E + 0.6380t + 0.0536T - 0.0312ET + 0.00746tT + 0.00024T^2 + 0.000168ET^2 + 0.0220EtC - 0.00784C^2, \quad (1)$$

where E is the species (zero means *C. laurifolius* and one means *P. pinaster*), T the temperature, t the time of exposure and C the concentration of ash.

An area under the ROC curve equal to 0.774, indicated that the logistic model had a good level of discrimination capacity.

At 1 min of exposure (Fig. 3), the maximum germination probabilities for *P. pinaster* were reached between 90 and 120 °C, and there were no significant differences with the remaining. In the case of *C. laurifolius*, these maximum germination rates were reached at slightly lower temperatures, 90–100 °C, with the greatest differences between these species appearing both at temperatures near the control and at the most extreme temperatures. The results also showed that, at this time of exposure, high ash concentration had a negative result on the germination of seeds of both species, especially *C. laurifolius*. If the exposure time was 5 min, the likelihood of germination decreased with temperature. In this case, and for *P. pinaster*, the germination rates rose when ash concentration increased, the opposite being true for *C. laurifolius*.

4. Discussion and conclusion

Our results for *P. pinaster* agree with those found by Núñez and Calvo (2000), in other *Pinus* species in that when temperature is equal to 150 °C or higher, the seeds are more sensitive to exposure time. It was found that at temperatures above 150 °C, and exposure time of 5 min, germination was zero, whereas at 1 min exposure and 150 °C, germination of *P. pinaster* seeds was greater than 60%. Thermal treatments at temperatures of 70, 90 and 110 °C, regardless of the time of exposure, do not have a significant effect on germination.

When exposure time is 1 min this effect is achieved at temperatures up to 170 °C. In contrast, when exposure time is 5 min, temperatures over 130 °C have a strong negative impact on seed germination, which is completely inhibited at temperatures over 150 °C.

Interspecific competition can modify stand structure after a wildfire. One of the main competitors of *P. pinaster* in Mediterranean areas is *C. laurifolius*. Like the majority of Mediterranean shrubs, many *Cistus* spp. produce a large number of seeds with a hard impermeable seed coat. Seeds are stored in soil seed banks and germinate massively after fires, as a strategy for recolonizing the burnt area. Problems in seedling recruitment for interspecific competition have been widely recorded elsewhere in Mediterranean pine

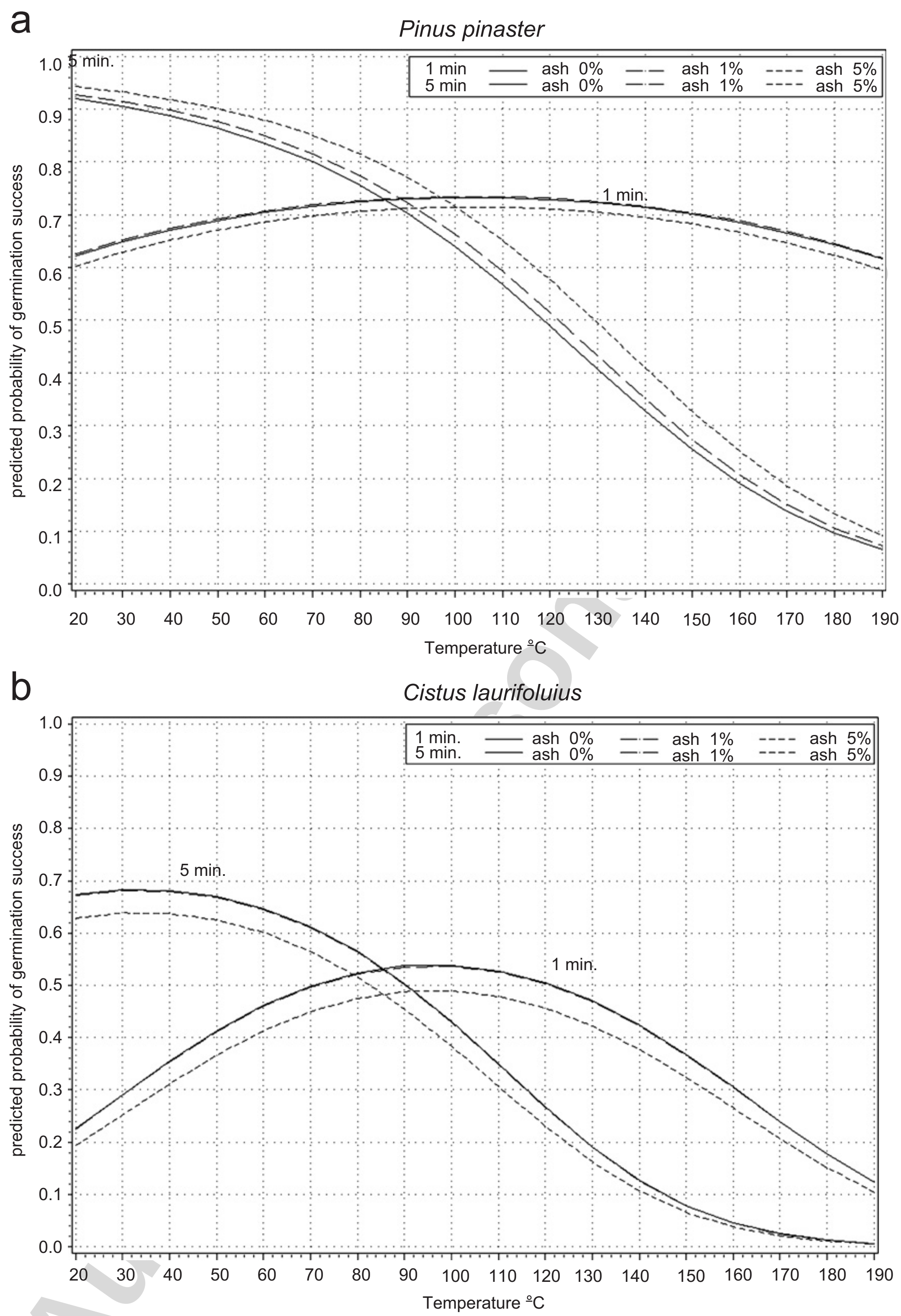

Fig. 3. Predictor curves to the probability of germination success to *Pinus pinaster* and *Cistus laurifolius* from logistic regression.

species (González-Martínez and Bravo, 2001). Our findings on germination response of *C. laurifolius* agree with previous work on this genus (Trabaud and Oustric, 1989, Valbuena et al., 1992).

Ash has been recorded as having an effect on the germination process after wildfires and on the subsequent development of seeds (Reyes and Casal, 1998, 2006); however, our experiments did not provide evidence that this was the case for *P. pinaster* and *C. laurifolius*.

Atmospheric models forecast, as a cronosequence of climate change, show a future increase on the frequency and severity of natural disturbances such as fires, pests, diseases, wind storms, join drought episodes and a general rise of temperatures in the Mediterranean Basin (Peñuelas et al., 2004). These new climate characteristics, coupled with the species composition and structure of Mediterranean-type forests, will make them especially susceptible to the propagation and recurrence of wildfire events (Sánchez-Palomares et al., 2003). Therefore, it is necessary to know the germinative response in the new possible scenario. Other works have studied the post germination behavior in arid and semiarid lands in different species (Kulkarni et al., 2007; Moreno and Oechel, 1991; Villalobos et al., 2002) to design the best strategy of conservation.

In this sense, in forest management, prescribed burning on mechanical site preparation could favor *P. pinaster* establishment by limiting competition. However, currently, prescribed fire has a limited use in Spanish forestry. In this study, we have determined which thermal treatments are effective in the regeneration of *P. pinaster*. The temperature range between 70 and 110 °C is effective for the germination of *P. pinaster*, regardless of exposure time. In rapid fires, the temperature may rise above 110 °C, or even as high as 190 °C. However, if the time of exposure is greater, satisfactory regeneration will only be achieved up to 130 °C, when success decreases progressively as the temperature increases.

In order to avoid *Cistus* competition, temperatures reached in prescribed burns should be kept under 90 °C, a temperature that permits good germination on *P. pinaster* and less germination in *C. laurifolius*.

Although for the time exposure studied *Cistus* germination did not surpass *Pinus* germination, further studies are needed before this hypothesis can be stated definitely because in other pines a drastic reduction of germination rate has been observed at exposure times of 10 or 15 min.

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References

Arianoutsou, M., Margaris, N.S., 1982. Phryganic (east mediterranean) ecosystens and fire. Ecol. Medit. VIII, 473–480.

Castro, J.F., Bento, J., Rego, F., 1990. Regeneration of *Pinus pinaster* forest after wildfire. In: Goldammer, J.G., Jenkins, M.J. (Eds.), Fire in Ecosystem Dynamics. SPB Academic Publishing, The hague, pp. 71–75.

Clark, D.L., Wilson, M.V., 1994. Heat treatment effects on seed bank species of an old-growth Douglas fir forest. Northwest Science 68 (1).

Come, D., 1970. Les obstacles á la germination Masson, Paris.

Gill, A.M., Groves, R.H., 1981. Fire regimes in heath-lands and their plant ecologic effects. In: Specht, R.L. (Ed.), Ecosystems of the World. Heathland and Related Scrublands, vol. 9B. Elservier, Amsterdam, pp. 61–84.

González-Martínez, S.C., Bravo, F., 2001. Density and population structure of the natural regeneration of Scots pine (*Pinus sylvestris* L.) in the High Ebro Basin (Northern Spain). Annals of Forest Science 58, 277–288.

Hanley, M.E., Fenner, M., 1998. Pre-germination temperature and the survivorship and onward growth of Mediterranean fire-following plant species. Acta Oecologica 19 (2), 181–187.

Hernando, C., Guijarro, M., Díez, C., 2000. Régimen térmico en troncos de *Pinus pinaster*: aplicación a las quemas prescritas. In: Vega, J.A., Vélez, R., (Eds.), Actas de la Reunión sobre Quemas Prescritas. Lourizán 1998. Sociedad española de Ciencias Forestales. Cuadernos de la S.E.C.F, No 9, 2000, pp. 75–89.

Kulkarni, M.G., Sparg, S.G., Van Staden, J., 2007. Germination and post-germination response of Acacia seeds to smoke-water and butenolide, a smoke-derived compound. Journal of Arid Environments 69 (1), 177–187.

Malanson, G.P., 1984. Intensity as a 3rd factor of disturbance regime and its effects on species diversity. Oikos 43, 411–413.

Montgomery, D.C., 1997. Design and Analysis of Experiments, fourth ed. Wiley, New York.

Montgomery, K.R., Stird, W.T., 1976. Regeneration of introduced species of *Cistus* (Cistaceae) after fire in Southern California. Madroño 23, 417–427.

Moreno, J.M., Oechel, W.C., 1991. Fire intensity effects on germination of shrubs and herbs in Southern California chaparral. Ecology 72, 1993–2004.

Neéman, G., Lavah, H., Izhaki, I., 1993. The effect of ash on the germination and early growth of shoots and roots of *Pinus*, *Cistus* and annuals. Seed Science and Technology 21, 339–349.

Núñez, R., Calvo, L., 2000. Effect of high temperatures on seed germination of *Pinus halepensis* and *Pinus sylvestris*. Forest Ecology and Management 131, 183–190.

Oria de Rueda, J.A., Díez, J., 2003. Los bosques de Castilla y León. Ámbito Ed. S.A. Valladolid. Spain.

Pérez, F., Pita, J.M., 1996. Ecofisiología de la germinación de las jaras (*Cistus* ssp.). Lecciones hipertextuales de botánica ⟨revista@quercus.es⟩.

Peñuelas, J., Filella, I., Zhang, X., Llorens, L., Ogaya, R., Lloret, F., Comas, P., Estiarte, M., Terradas, J., 2004. Complex spatiotemporal phenological shifts as a response to raingall changes. New Phytologist 161, 837–846.

Reyes, O., Casal, M., 1998. Germination of *Pinus pinaster*, *Pinus radiata* and *Eucaliptus globulus* in relation to the amount of ash produced in forest fires 1997. Annals of Forest Science 55, 837–845.

Reyes, O., Casal, M., 2006. Seed germination of *Quercus robur*, *Q. pyrenaica* and *Q. ilex* and the effects of smoke, heat, ash and charcoal. Annals of Forest Science 63, 205–212.

Sánchez-Palomares, O., Rubio, A., Blanco, A., Elena, R., Gómez, V., 2003. Autoecología paramétrica de los hayedos de Castilla y León. Investigación Agraria. Sistemas y Recursos Forestales 12 (1), 87–110.

SAS Institute Inc., 2001. SAS/STATTM User's Guide, Relase 8.2, Cary, NC, USA.

Sousa, WP., 1984. The role of disturbance in natural communities. Annual Review of Ecology and Systematics 15, 353–391.

Tapias, R., Gil, L., Fuentes-Utrilla, P., Pardos, J.A., 2001. Canopy seed bank in Mediterranean pines of South-eastern Spain: a comparison between *Pinus halepensis* Mill., *P. pinaster* Ait., *P. nigra* Arn., and *P. pinea* L. Journal of Ecology 89, 629–638.

Thanos, C.A., Marcou, S., Christodoulakis, D., Yannitsaros, A., 1989. Early post fire regeneration in *Pinus brutia* forest ecosystems of Samos island (Greece). Acta Oecologica, Oecologia Plantarum 10 (1), 79–94.

Thomas, P.A., Wein, R.W., 1990. Jack pine establishment on ash from wood and organic soil. Canadian Forest Research 20, 1926–1932.

Trabaud, L., 1979. Etude du comportement du feu dans la Garrige de Chene kermès à partir des températures et des vitesses de propagation. Annales des Sciencies Forestières 36 (1), (13:38).

Trabaud, L., Oustric, J., 1989. Heat requirements for seed germiantion of 3 *Cistus* species in the garrigue of Southern France. Flora 183, 321–325.

Trabaud, L., Michels, C., Grosman, J., 1985. Recovery of burnt *Pinus halepensis* Mill., forest. II Pine reconstitution after wildfire. Forest Ecology and Management 13, 167–179.

Valbuena, L., Tárrega, R., Luis, E., 1992. Influence of heat on seed germination of *Cistus laurifolius* and *Cistus ladanifer*. International Journal of Wildland Fire 2, 15–20.

Vega, J.A., 2000. Resistencia vegetativa ante el fuego a través de la historia de los incendios. In: Vélez, R. (Ed.), La Defensa Contra Incendios Forestales: Fundamentos y Experiencias. Mc Graw Hill Interamericana de España, S.A.U. Madrid, pp. 4.66–4.85.

Villalobos, A.E., Peláez, D.V., Bóo, R.M., Mayor, M.D., Elia, O.R., 2002. Effect of high temperatures on seed germination of Prosopis caldenia Burk. Journal of Arid Environments 52 (3), 371–378.

Viullemin, J., Bulard, C., 1981. Ecophysiologie de la germination de *Cistus monspelliensis* L. Naturalia Monspeliensis. Série Botanique 46, 1–11.

Warburg, E.F., 1980. Cistus. In: Tutin, T.G., Heywood, V.H., Burges, W.A., Moore, D.M., Valentine, D.H., Walters, S.M., Webb, D.A. (Eds.), Flora Europaea, vol. II. Cambridge University Press, London, UK, pp. 282–284.